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THE FORMATION AND EARLY DIFFERENTIATION OF SEA URCHIN GONADS

MARGARET S. HOUK AND RALPH T. HINEGARDNER

Division of Natural Sciences, University of California, Santa Cruz, California 95064

Despite extensive use of sea urchin gametes in developmental biology, few modern studies deal with the origins and differentiation of the gametes from the primary germ cells (PGCs). All published studies are from the last century or the early part of this one, the major ones being those by Hamann (1887); Prouho (1887); Cuenot (1891); Russo (1894); and MacBride (1903). This and other work on gonad development has been summarized by Delavault (1966). Most authors agree that the gonadal or genital primordium, variously called the genital rudiment, genital cord, or sexual bud, is identifiable soon after metamorphosis from pluteus larva to juvenile urchin as an accumulation of large cells located in the dorsal mesentary. This mesentary, which forms from the apposition of the walls of the left and right posterior coeloms of the larva (the somatocoels of the adult), adheres to the aboral body wall in the vicinity of the madreporite and extends into the main body cavity to form the boundary between the left and right somatocoels. The dorsal mesentary also supports the stone canal, axial organ, and parts of the gut. Published studies indicate that the genital primordium later spreads circumferentially around the inside aboral surface and eventually forms a circular cord of cells, the genital rachis, which competely encircles the periproct. The gonads differentiate from five swellings on the rachis, with one gonad forming in each interradius.

We initiated this study to determine the accuracy of the earlier observations and to expand upon them through electron microscopy. We have confirmed the location of the gonadal primordium in the dorsal mesentery and its subsequent development into genital rachis and gonads. We differ, however, from most previous investigators in our interpretation of some features of the process. Our ultrastructural examination of the gonadal primordium sheds additional light not only on the origin of the gametes, but on the origin and differentation of the gonadal accessory cells (sometimes called the nutritive phagocytes).

Materials and Methods

Selection of material

The sea urchins we used were out-crossed laboratory-raised individuals of the species *Lytechinus pictus*, grown according to the methods of Hinegardner(1969). Juvenile urchins selected for tissue sectioning ranged from 0.3 to 6.0 mm in outer diameter and in age from 3 days post metamorphosis to 8 months post metamorphosis. We have been able to identify the genital cells in all stages up to the time when recognizable eggs and sperm are found.

Preparation for light microscopy

Juvenile urchins were fixed in ethanol-acetic acid, 3:1, and dehydrated in an ethanol series. Urchins under 3 mm in diameter were embedded for serial

sectioning first in celloidin and then in paraffin according to the method of Akesson (1961). Larger juveniles were embedded directly in paraffin. Five μm serial sections were used. After removal of the paraffin with xylene, sections were passed from 100 to 90% ethanol and stained for 20 sec in Unna-Pappenheim methyl-green pyronin stain (Gurr, 1965). They were then washed in water for 1 min, blotted to remove excess water, and passed directly to 100% ethanol for 10 sec of agitation. Rapid passage through water and ethanol was necessary to prevent loss of stain. Cell counts at various growth stages were made from *camera lucida* drawings using semi-transparent paper so that tracings could be overlaid for comparison. For each individual in which germ cells were counted, all sections containing any germ material were traced and compared with adjacent sections to avoid counting the same cell more than once.

Preparation for transmission electron microscopy

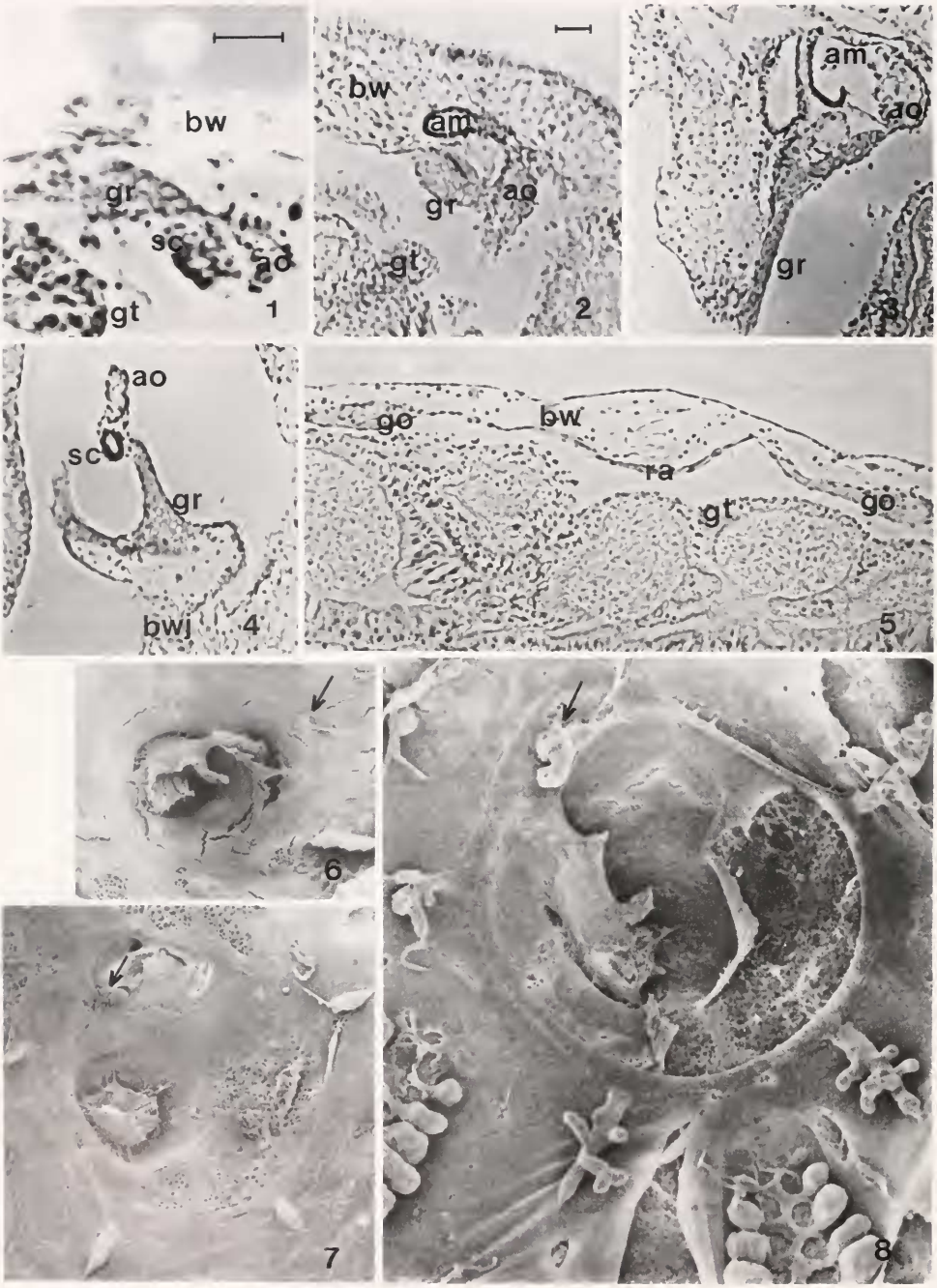
Urchins were prepared for fixation by several methods depending on their size. Gonads from mature individuals (> 10 mm in diameter) were excised from the body cavity prior to fixation. Juvenile urchins were bisected through their largest diameter. The aboral half, containing the germ cells, was then inverted to form a "bowl" and flushed several times with fixative prior to immersion in fresh fixative. The desired tissues were then dissected from the urchin. Juveniles under 1.5 mm were fixed whole.

Most material was fixed in 1.5% glutaraldehyde in sea water for 1 hr. All manipulations were carried out at pH 7.0–7.6 and at 4°C. A few samples were fixed according to the method of Bal *et al.* (1968). Tissues were next washed in several changes of sea water and post-fixed for 2–2.5 hr in 1% osmium tetroxide in sea water. Dehydration was carried out in an acetone series. Tissues were embedded in Spurr's resin (Spurr, 1969). Material from early juvenile stages had to be decalcified because the minute germinal primordia could not be removed by dissection. Decalcification was carried out on material fixed overnight in 1.5% glutaraldehyde followed by osmium tetroxide as described above. Samples were either decalcified in ascorbic acid according to the method of Dietrich and Fontaine (1975) or in 5% EGTA in 0.1 M sodium phosphate buffer at pH 7 for 1 hr.

Blocks of tissue were first sectioned (0.5 μm) for purposes of orientation. These thick sections were stained for light microscopy with 1% toluidine blue in 1% sodium borate. Thin sections were cut with a Porter-Blum MT-2 ultra-microtome using a glass knife. Sections were stained for 30 min with Millipore filtered 2% uranyl acetate and 5 min with Reynolds' lead citrate (Reynolds, 1963). The electron microscope was a JEOL GEM 100B.

Preparation for scanning electron microscopy

Juvenile urchins ranging from 3 to 7 mm in diameter were prepared for scanning electron microscopy by bisecting the specimen equatorially slightly on the aboral side of the greatest diameter. The aboral part was flooded with 1.5% glutaralde-hyde in sea water and any remaining pieces of gut carefully dissected out under a dissecting microscope. Specimens were dehydrated in an ethanol series, substituted with amyl acetate, then critical-point dried in carbon dioxide (Anderson, 1951), coated with carbon and gold and examined with a JEOL JSM-2 scanning electron microscope.



FIGURES 1–5. Paraffin sections through the germinal tissues of juvenile sea urchins. Figure 1—3-day-old juvenile 0.3 mm in diameter. Figure 2—Month-old juvenile 1.0 mm in diameter. Figure 3—Three-month-old juvenile, 2.0 mm in diameter, sectioned at a 45° angle to the oral-aboral axis. Figure 4—Three-month-old juvenile, 2.0 mm in diameter, sectioned circumferentially. The genital primordium *gr* is suspended between the aboral body wall *bw* and stone canal *sc* and closely associated with the axial organ *ao* near where the gut *gt*

TABLE I

Number of primordial germ cells present in the juvenile sea urchin at different post-metamorphosis ages and test diameters

Days past metamorphosis	Outer diameter of test (mm)	No. urchins counted	Average no. germ cells ± SD
1–3	0.3	5	20.8 ± 5.4
29	0.68	1	21
29	0.85	3	36.0 ± 5.3
29	1.0	3	29.33 ± 3.8
29	1.2	3	60.3 ± 12.0
93	1.5	1	60
93	2.0	2	101.0 ± 26.9
Unknown	2.7	2	1000 approx.

RESULTS

Differentiation prior to gonad formation

In the newly metamorphosed sea urchin, the primordial germ cells (PGCs) of the genital primordium are identifiable in serially sectioned material as a group of pyrinophilic cells with large (5 μm) nuclei containing one or two nucleoli. The PGCs remain suspended in the mesentery between the stone canal and the inner surface of the aboral wall throughout early juvenile development (Fig. 1). By the time the urchin reaches a diameter of 1 mm (approximately 1 month after metamorphosis), the germ cells have clustered into a distinct group of cells within the germinal primordium (Fig. 2), and are surrounded by a layer of squamous epithelium originating from the mesentery. No coelomic cavity has been identified in association with this group of cells.

Two types of cells, as determined by nuclear morphology, can be found in the genital primordium. The first are the primary germ cells found in the mesentery at the time of metamorphosis. The other type, which we call the presumptive accessory cells, has a nucleus of about 2.5 μm in diameter that stains more heavily than the first type with methyl-green. These cells were not seen in the dorsal mesentery of newly metamorphosed urchins, and their origin is not known.

We found the size of juvenile urchins, rather than their post-metamorphic age, to be the more reliable indicator of the degree of differentiation of the genital tissues (Table I), and therefore include test diameter as an indication of the developmental

meets the anus. *am* = ampulla of the left anterior coelom. *bwj* = junction of projecting part of body wall with the main body wall. Figure 5 shows the aboral region of a juvenile 2.5 mm in diameter. Two of the gonadal primordia *go* are connected by part of the genital rachis *ra*. Bars = 10 μm. Figures 2–5 are at the same magnification.

FIGURES 6–8. Scanning electron micrographs of the inner perianal region of juvenile sea urchins. Most of the gut has been removed. Figure 6 shows an individual 1.5 mm in diameter. The torn edges of the intestine surround the anal area at the left corner, while the axial organ and attached dorsal mesentery containing the genital primordium appear at upper right (arrow). Figure 7 depicts an urchin 3.5 mm in diameter with unbranched gonads, four of which are arranged in a circle, one each right and left, and two at the bottom of the figure. The axial organ appears just above the gut in the upper left corner (arrow). Figure 8 shows the gonads of an urchin 5.0 mm in diameter. Four gonads and axial organ (arrow) are shown, oriented as in Figure 7. All three figures are at the same approximate magnification (× 55–60). Field width for 6 is 1.04 mm, for Figure 7 is 1.65 mm, and for Figure 8, 2.02 mm.

stage of our material. In urchins as small as 1.5 mm in diameter, the genital primordium has already begun to broaden and elongate within the dorsal mesentery due to an increase in the number of PGCs (Table I). At this stage genital cells occupy most of the dorsal region of that mesentery. Subsequent gonad development is easiest to understand by visualizing the primordium as something like a letter I lying in the mesentery. As the urchin grows the PGCs migrate to the top of the I, where the dorsal mesentery joins the dorsal body wall. The PGCs then begin to migrate laterally, converting the I to a T-shaped structure. The arms of the T gradually elongate and its stem shortens. Evenutally the stem disappears and the elongating arms form a ring around the periproct. This ring is called the genital rachis (Hamann, 1887). Its diameter is about 1/4 the diameter of the urchin.

Figures 3 and 4 illustrate sections from urchins 2.0 mm in diameter, which have just begun to form a rachis. The section in Figure 3 is approximately perpendicular to the oral-aboral axis and shows the location of the band of genital tissue relative to the left anterior coelom (axocoel), the axial organ, and the stone canal. The rachis lies along part of the body wall which projects into the coelomic space due to the presence of the axocoel and its associated structures. Figure 4 is oriented approximately 45° to Figure 3 and shows the arms of the T-shaped genital primordium. There is a continuum of cells throughout the primordium, with no separation between those beginning to form the rachis and those still within the mesentery. The genital primordium in Figure 4 appears to be connected to the main body wall by only a narrow piece of tissue, but this appearance is due to the angle of the section.

After the rachis has formed, five thickenings appear in it, one in each interradius. These eventually contain most of the PGCs and ultimately give rise to the five gonads. Two such forming gonads and a large portion of the genital rachis, from an urchin 2.5 mm in diameter, are shown in Figure 5. Both the rachis and the developing gonads are surrounded by a layer of epithelium which seems to be derived from the epithelium for the mesentery in which the genital primordium was first found, and thus to be coelomic in origin. Both cell types found in the genital primordium are present in the rachis and in the developing gonads. In larger urchins, with differentiated gonads, the solid cord of PGCs in the rachis has disappeared.

Differentiation of the gonads

No gonads are visible in urchins 1.5 mm in diameter (Fig. 6). Unbranched gonads are present in a 3.5-mm-diameter urchin (Fig. 7). A raised circular area on the aboral wall connects the bases of the gonads, indicating the presence of the genital rachis. Figure 8 shows this area of an animal 5.0 mm in diameter. The gonads have branched several times. That a rachis still exists, even in an individual in which all the germ cells have undoubtedly migrated into the gonads, can be seen in the upper right portion of Figure 8, where the rachis has torn free from the underlying epithelium. We can therefore corroborate the persistence of the now empty rachis as the aboral ring canal, as reported by Cuenot (1891) and Russo (1894). Further branching continues to occur as the gonads grow, until the aboral part of the coelom contains a mass of branching tubules. Each gonad is surrounded by an epithelial layer continuous with the lining of the perivisceral coelom. Besides separating the gonad from the main body cavity, this cell layer

forms mesenteries which attach it to the aboral body wall. No gonoduct is present at this time.

Sexual differentiation of the gonad is first apparent with the presence of recognizable oocytes and spermatids. These appear at about the same time or shortly after the gonads begin to branch. By the time male urchins reach a diameter of 5 mm their gonads contain mature spermatozoa in the central cavity, but they usually cannot be induced to spawn. Females 5–6 mm in diameter have previtellogenic oocytes up to 25 μm in diameter (mature eggs are about 110 μm in diameter). The structure of the female gonad at this stage does not differ substantially from that of published descriptions of the mature gonad of other species (Holland and Giese, 1965; Chatlynne, 1969; Davis, 1971; and Pearse, 1969a, b). A gonoduct leads from the central cavity of the gonad to the outer wall of the test. It consists of a single layer of cuboidal cells of unknown origin.

Ultrastructure of the genital tissues

Three stages in the development of the juvenile urchins were selected for ultrastructural studies. These stages were: 1) the genital primordium of newly metamorphosed urchins (0.5 mm in diameter), 2) the older genital primordium, in which the PGCs are rapidly proliferating (1.5 mm), and 3) a sexually undifferentiated gonad (test diameter 3.5 mm). Gonads from sexually differentiated urchins were also studied for comparative purposes and to check our results against published studies where different species and fixation procedures were used.

1. Genital primordium of newly metamorphosed urchin. A tangential section through the genital primordium of a sea urchin with test 0.5 mm in diameter (3 weeks after metamorphosis) is shown in Figure 9. The genital primordium is roughly 33 μm across and is bounded on the outside by an epithelium of ciliated squamous cells. A thick connective-tissue layer lies beneath the epithelium and contains scattered phagocytic cells. The connective-tissue layer is not strictly separated from the underlying cells, and fibrous material similar to that in the connective tissue is often found between these cells. The genital primordium is separated from the body wall by the epithelium of the mesentery and by the peritoneum of the somatocoel. A pronounced basement lamina is found underlying the epithelial cells. The mesentery containing the genital primordium is attached to the body wall *via* the epithelial layers at the right of the figure.

Two cell types are found within the genital primordium. The first are elongate, smooth surfaced, rounded in cross section, and have a round to oval nucleus containing a relatively electron-transparent nuclear matrix. The nucleus is 5–6 μm in diameter. These cells correspond to the primordial germ cells seen with the light microscope. A fibrillar material, presumably chromatin, is fairly evenly distributed within the nucleus. Dense granules about 60 nm in diameter are interspersed among the chromatin material (Fig. 10). The cytoplasm contains the usual cellular organelles, most of which are concentrated in the tapered ends of the cells. Membrane-bound vesicles as large as 0.9 μm in diameter and containing an electron-dense amorphous material are also present (Fig. 9). These are not seen in the larval material we have studied nor in later stages.

Associated with the mitochondria, and sometimes apparently touching their outer membranes, are 100 nm granules containing a dense fibrillo-granular material. These are probably identical to the granules found by Longo and Anderson (1969) in sea urchin spermatogonia. The granules are sometimes clustered (Fig. 11).

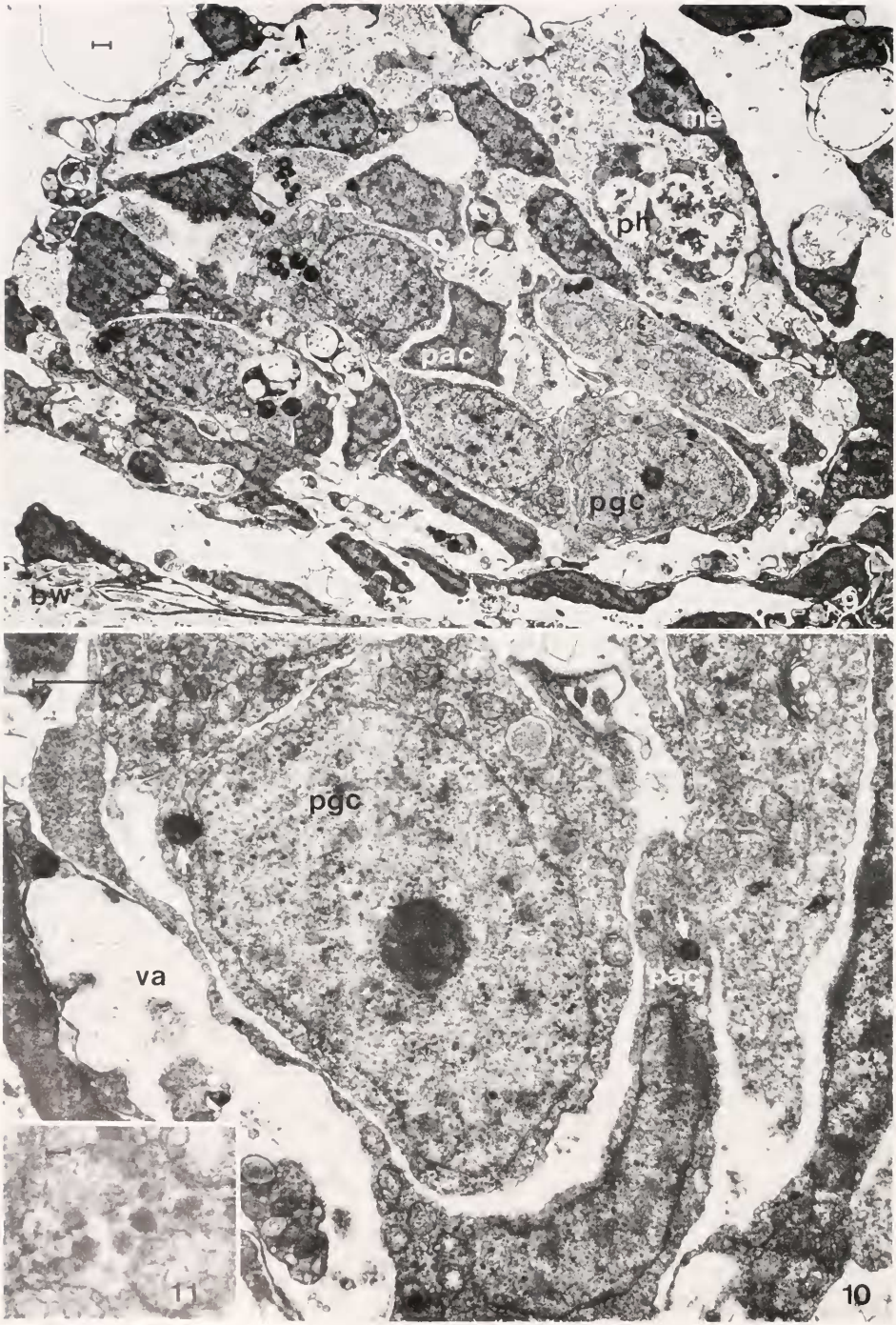


FIGURE 9. Section through the genital primordium of an urchin 0.5 mm in diameter showing the mesentary epithelium *mc*, with its underlying basement lamella (arrow), primordial germ cells *pgc*, presumptive accessory cells *pac*, and a phagocytic cell *ph*. The epithelium of the mesentery joins that of the body wall *bw* at the lower right hand side of the figure. Bar = 1 μm.

They are common in these cells as well as those in later stages. Since they seem to be specific to the gamete-forming cells, we will refer to them as *goniosomes*. Amorphous electron-dense material similar to that found in germ cells of other organisms (Eddy, 1975) is also present in the cytoplasm of PGCs.

The cells that we are calling primary germ cells share a number of characteristics with gonial cells, of which they are certainly the precursors. These include: nuclear morphology; the general appearance and contents of the cytoplasm, including goniosomes; and staining characteristics using methyl-green pyronin and toluidine blue.

The second cell type is almost certainly the precursor of the accessory cells of the gonad, which it resembles in both shape and nuclear morphology. These accessory-precursor cells are smaller than the PGCs and are generally angular in cross section, with many elongate cellular processes (Figs. 9, 10). The nucleus is also elongate and irregular in outline. Its matrix is more electron-dense than is the nucleus of the PGC, and areas of condensed chromatin are obvious. Small dark 60 nm granules are present here as they were in the PGCs, and cytoplasmic inclusions are similar. Structures which look like the 100 nm goniosomes common in the PGC have not been found in these cells. The accessory cell primordium also contains a large electron-transparent vacuole (Fig. 10) similar to that found in the accessory cell of the mature gonad (Holland and Giese, 1965). Flagellar cross-sections among the cells of the genital primordium indicate that PGCs and/or accessory-cell precursors are flagellated.

2. Genital primordium with rapidly proliferating PGCs. The genital primordium cells of a 1.5 mm urchin are similar to those of the younger animal (Fig. 12). The PGCs have the same general appearance except for the absence of the large membrane-bound vesicles filled with electron-dense material. Mitotic cells can now be found. Otherwise, the PGCs are unchanged.

The accessory-cell primordia have now become obvious accessory-cell precursors. The angular nucleus is surrounded by a very thin layer of cytoplasm (Fig. 12). Extensive processes containing large numbers of membrane-bound lipid-like vesicles, similar to those found in the accessory cells of mature gonads, can be seen extending between the PGCs. Glycogen granules, which are abundant in the accessory cells of the mature gonads, are often present in the cellular extensions. The vacuole has elongated. Nuclear morphology remains relatively unchanged and is similar to that of accessory cells of the mature gonad. Flagellar cross-sections are present among these cells.

3. Sexually undifferentiated gonads. A cross section of an unbranched gonad from a 3.5-mm-diameter urchin is shown in Figure 13. A many-layered gonad wall surrounds the PGCs. From outside to inside these layers consist of flagellated coelomic epithelium, a connective-tissue layer, a fluid-filled space, a musculoepithelial layer (also flagellated), and the germinal epithelium. This order is identical to that of the layers in the wall of the mature gonad (Kawaguti, 1965; Davis, 1971). The outer epithelium is continuous with the lining of the perivisceral coelom.

The PGCs dominate the outer germinal layer, and are arranged two or three cells thick. They are closely packed, adhering by means of junctional complexes

FIGURE 10. A primordial germ cell *pgc* in an urchin 0.5 mm in diameter is partially surrounded by an accessory cell *pac*. Both cells contain vesicles with electron dense material (arrows). The pac also has a large vacuole *va*. Bar = 1 μm.

FIGURE 11. Goniosome cluster from a PGC at the same stage shown in Figures 9 and 10. Bar = 0.1 μm.

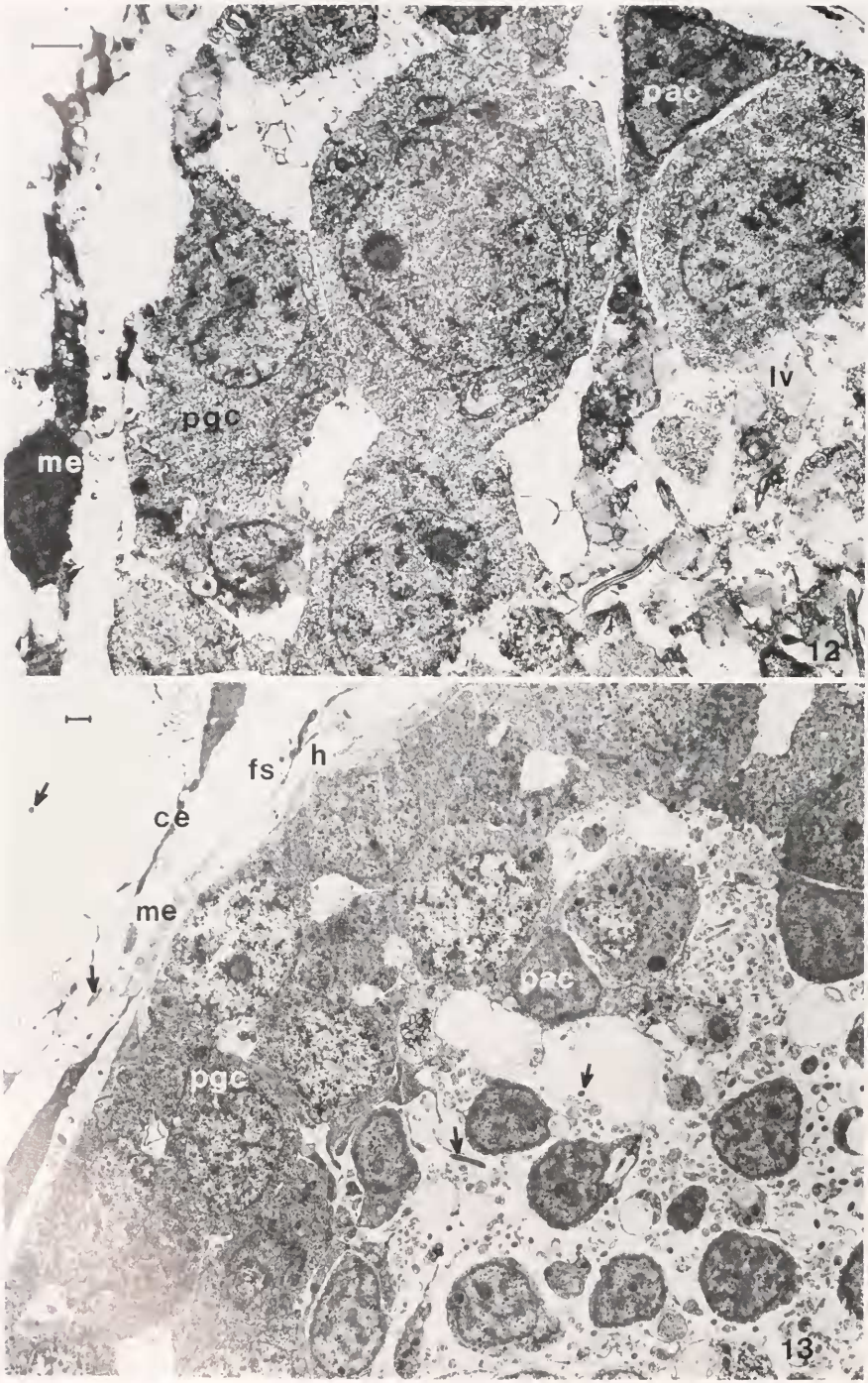


FIGURE 12. Cross section through the genital primordium of an urchin 1.5 mm in diameter, showing primordial germ cells *pgc* and presumptive accessory cells *pac* and the epithelium of the dorsal mesentary *me*. The PACs have long cellular processes containing numerous vesicles filled with lipid-like material *lv*. Flagellar longitudinal sections can be seen in the center portion of the lumen. Bar = 1 μm.

(Fig. 14) and frequently have long cell processes. The nuclei are round and exhibit various degrees of chromosomal condensation, indicative of ongoing mitotic activity. Up to three nucleoli have been seen in a PGC nucleus at this stage. The nucleoli are eccentric and have components with two different degrees of opacity to electrons. The dense cortical part surrounds a less dense fibrillar component. Nuclear pores with annuli are present.

Ribosomes are numerous in the cytoplasm, but endoplasmic reticulum is sparse. Mitochondria, Golgi, and a pair of centrioles are located mainly at the poles of the oval cell. The goniosomes that were described for the genital primordium PGCs are much more numerous at this stage and are scattered among the mitochondria and sometimes initimately associated with them (Fig. 15). Other goniosomes are grouped together in regular arrays (Fig. 14).

A loosely arranged layer of accessory cells underlies the germinal cells and extends into the central cavity of the gonad. Numerous cell processes, and occassionally, phagocytic cells similar to the one illustrated in Figure 9, are interspersed with these cells. The accessory cells are highly variable in shape and have many cell processes and a large vacuole (Fig. 16). The nuclei look like those of the accessory-cell precursors. Along with the usual organelles, the cytoplasm contains lysosomal-like vesicles identical to those so abundant in the accessory cells of the mature gonad. Glycogen granules and lipid vesicles are more abundant than in earlier stages. Large parts of the cytoplasm appear empty, which is also true of accessory cells of the mature gonad (Verhey and Moyer, 1967). The accessory cells at this stage are smaller than those of the mature gonad and are attached to each other and to the adjacent PGCs via tight junctions (Fig. 16). Sometimes accessory cells can be found completely encircling a PGC. Striated rootlets and flagellar bases have been seen in both the PGCs and the accessory cells, and flagellar cross sections are common in the gonad cavity and between cells (Fig. 13).

4. Sexual differentiation of the gonads. We examined the gonads of urchins 3.3–6.0 mm in diameter to establish a developmental continuum between the younger stages concentrated upon in this study and the ultrastructure of mature gonads as described in published accounts. The earliest indication of sexual differentiation was seen in a female urchin 3 mm in diameter with a gonad that had just begun to form branches. The wall of the gonads differed from sexually undifferentiated stages only in a more extensive development of the muscle fibers of the inner epithelium. Most of the PGCs adhere tightly to each other and are the same size as those of the undifferentiated gonad. However, there are a number of larger cells (10–20 μm in diameter) which have lost their close contacts with their neighbors and have become rounded. One such cell is pictured in Figure 17. The circular nucleus is 7 μm in diameter and centrally located with a single nucleolus. The cytoplasm has inclusions similar to those of the PGC but more evenly distributed. Goniosomes are present, often associated with mitochondria.

Older individuals (5.0 mm in diameter) show definite sexual differentiation. The solid layer of germ cells apparent in sexually undifferentiated gonads is no longer present, and clusters of gonia are separated from each other by accessory cell processes. In young ovaries a large part of the germinal layer consists of a

FIGURE 13. Cross section through the unbranched, sexually undifferentiated urchin 3.5 mm in diameter showing the outer coelomic epithelium *ce* and the muscular epithelium *me* of the gonad wall. The epithelium layers are separated by a fluid filled space *fs*, and a haemal lacuna *h* underlies the muscular epithelium. PGCs form a germinal epithelium next to the haemocoel, while PACs are in and near the central lumen. Ciliary cross sections of outer epithelium, muscular epithelium, and accessory cells are indicated by arrows. Bar = 1 μm.

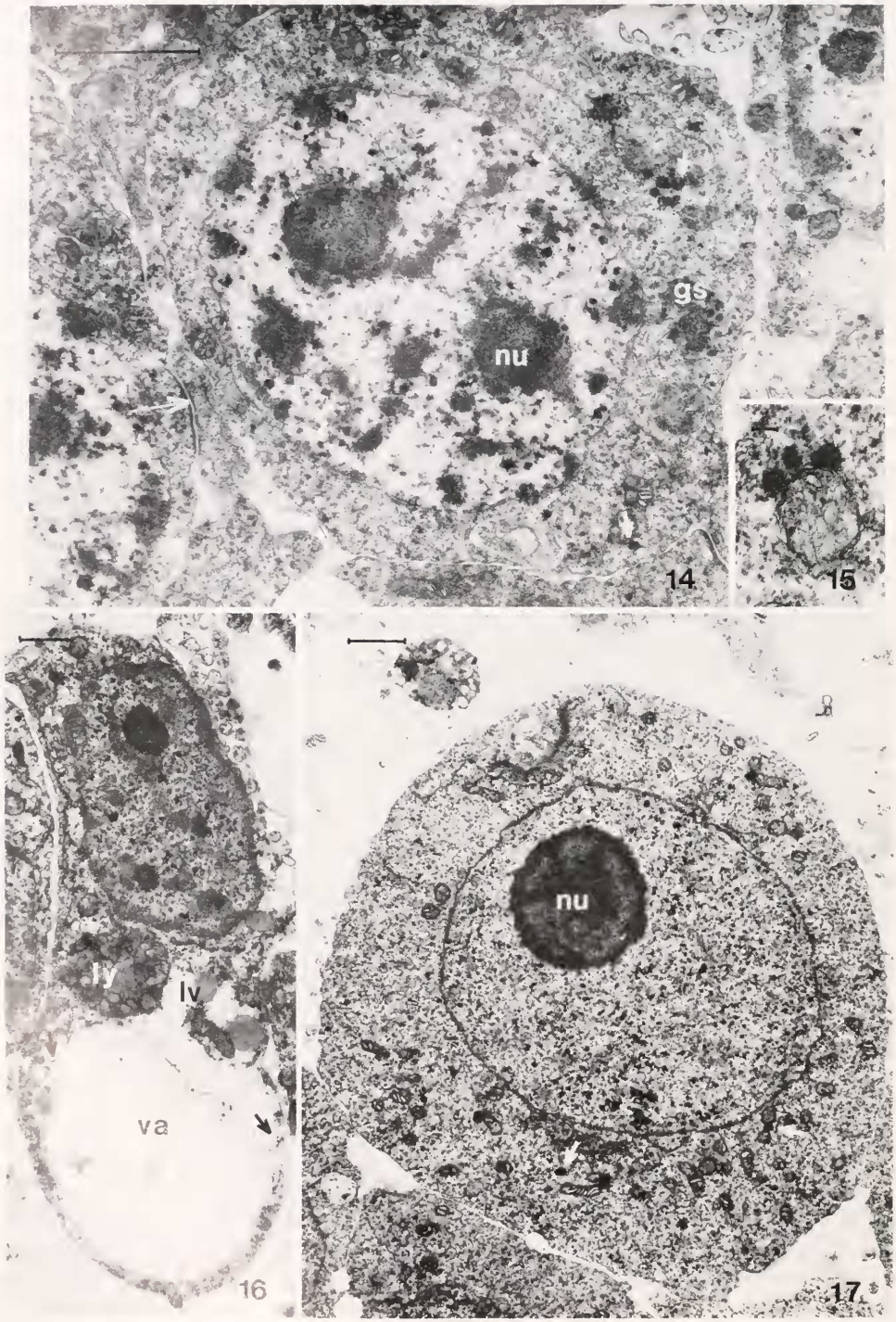


FIGURE 14. A primordial germ cell of the unbranched gonad. Two nucleoli *nu* are present. A cluster of goniosomes *gs* and unclustered goniosomes (short arrows) are in cytoplasm. The PGC adheres to its neighbors by junctional complexes (long arrows). Bar = 1 μm.

single layer of previtellogenic oocytes. Oogonial clusters are widely separated and extend from the gonad wall well into the lumen. Oogonia resemble PGCs in their nuclear morphology and cytoplasmic inclusions, but have a pronounced polarity, with the mitochondria and goniosomes clustered at one end of the oval cell. Polarity seems to be oriented randomly with respect to the ovary wall. Oocytes similar in size and shape to the one pictured in Figure 7 are occasionally seen near the lumen of the ovary. Most of the oocytes, however, are larger, about 25 μm in diameter, and are located in the single layer next to the ovarian wall. The accessory cells resemble those of the mature gonad. Oogenesis has been described in a number of species of sea urchins (*e.g.*, Verhey and Moyer, 1967; Bal *et al.*, 1969; Millonig *et al.*, 1968; Chatlynne, 1972).

Testes in these young urchins are organized similarly to those of mature males. All individuals which could be identified as males already had mature sperm present in their testes lumina. Presumably the spermatogenic cycle is so rapid (less than 12 days in gonads of some species of urchins; Holland and Giese, 1965) that testes containing spermatids and no mature sperm would be hard to find. Spermatogonia are clustered against the wall of the testes and resemble oogonia and PGCs. Spermatogenesis is well described in Longo and Anderson (1969).

Discussion

While most authors agree that the gonads of echinoderms differentiate from a group of cells located in the dorsal mesentery of the newly metamorphosed juvenile, the origin of the cells is disputed. Cuenot (1891) and MacBride (1903) believed that the genital primordia of asteroids, ophiuroids, and echinoids have common origins with the axial organs. In fact, MacBride refers to the axial organ as the genital stolon. Prouho (1887) and Russo (1894) described the genital primordium of sea urchins as arising from the wall of the left anterior coelom independently of the axial organ. MacBride (1903) also proposed an epithelial origin for the germinal cells of sea urchins, since he believed that the genital stolon, from which both the genital primordium and the axial organ supposedly differentiated, arose from the wall of the left posterior coelom of the newly metamorphosed urchin. More recent work by Delavault (1966) on the sea star *Asterina gibbosa* indicates that at least in sea stars, the location of the germ cells in the dorsal mesentery may be secondary and that the genital primordium may descend from mesenchyme cells which migrate to the mesentery during late larval development. Our study of newly metamorphosed juveniles of *Lytechinus pictus* shows that the germ cells are already present in the dorsal mesentery at the time of metamorphosis as a cluster of cells distinct from the axial organ and not connected to the wall of the coelom. Their ultrastructure resembles that of the mesenchymal cells. If these cells do originate from the coelomic epithelium, it must happen during larval development, contrary to MacBride's description for *Echinus esculentus* (1903), or Cuenot's for *Paracentrotus lividus* (1891). While their location and ultrastructural appearance sug-

Figure 15. Goniosomes closely associated with a mitochondrion in a PGC of the sexually undifferentiated urchin. Bar = 0.1 μm.

Figure 16. Presumptive accessory cell in unbranched gonad with lipid vesicles *lv*, lysosomal-like vesicles *ly*, large vacuole *va*, and glycogen granules (arrows). Bar = 0.1 μm.

Figure 17. Large oocyte in branched gonad of urchin 3.3 mm in diameter. The single nucleolus *nu* is differentiated into two regions of different opacity to electrons. Goniosomes are present in the cytoplasm (arrows). Bar = 1 μm.

gest a mesenchymal origin for the germ cells, careful study of larval development will be necessary to decide this question.

In other classes of echinoderms, particularly the asteroids, the genital rachis is described as developing from a vesicle which buds off the peritoneum of the left somatocoel and surrounds the germ cells. The space within the vesicle is derived from the coelom. In asteroids this space persists in the wall of the gonad and remains connected with the coelom (Delage and Hérouard, 1903). Such a vesicle does not appear in *Lytechinus pictus*. Rather, the germ cells begin to divide and to travel within the dorsal mesentary to the aboral body wall, extending out into a ring continuous with the mass of cells in the dorsal mesentary. This continuity indicates that the germ cells in the rachis must be between the peritoneum of the coelom and the body wall. The outer covering of both the primordium in the mesentery and the gonad wall is the peritoneal epithelium. The inner musculo-epithelial layer of the gonad wall is not present in the genital primordium stage. The origin of this secondary epithelial layer is not explained by our investigation. The layer appears to be present in the rachis, but ultrastructural examination would be necessary to determine this with certainty. The space between the two epithelia is probably a schizocoel, as suggested by Hamann (1887).

The accessory cells, also called nutritive phagocytes, have been studied in the mature gonad by several authors (Holland and Giese, 1965; Verhey and Moyer, 1967; Masuda and Dan, 1977). These cells are present in both sexes of the adult and change in size and internal constitution during the gametogenic cycle, giving rise to the hypothesis that they are involved in recycling nutrients from phagocytized gametes (Holland and Giese, 1965; Pearse, 1969a, b). Various origins for these cells have been suggested. Some authors have claimed that they originate from the same cells in the gonads as the gonia and therefore fit the classical definition of nurse cells (Miller and Smith, 1931). In our study we have seen that accessory cell primordia are present in the genital primordium soon after metamorphosis and migrate with the PGCs from the genital primordium through the rachis into the gonad. Differentiation into recognizable accessory cells consists primarily of the accumulation of cytoplasmic inclusions such as glycogen granules, lipid-like vesicles, and lysozomal-like vesicles. It begins while the cells are still in the genital primordium. This gradual accumulation of the cytoplasmic structures characteristic of accessory cells leaves little doubt that these cells differentiate from the small-nucleus cells of the genital primordium.

In young juveniles 1 mm in diameter, the cytoplasm of both the PGCs and the presumptive accessory cells contains membrane-bound vesicles filled with an electron-dense material which resembles yolk. These vesicles are seldom seen in either cell type at later stages. Possibly they are diluted as the cells divide. The presence of yolk in the PGCs of sea urchins is a reasonable suggestion, since PGCs of anuran amphibians are known to contain yolk platelets (Blackler, 1958). If these vesicles prove to be generally present in PGCs of larvae as well, they may be useful as cytoplasmic markers for tracing the germ cells back through larval development. While the PGCs and the presumptive accessory cells are distinct from each other in all the stages covered by this study, the presence of yolk-like structures in both cell types suggests they may have a common embryological origin.

While the PGCs share some features with the accessory cells, they are much closer in appearance to gonia. They have similar nuclear morphology, general cell shape, and cytoplasmic inclusions, especially the 100-nm goniosomes which were

found at all stages examined and only in germ-line cells. Similar granules have been found in the germ lines of insects (Mahowald, 1971) and amphibia (Kalt, 1973; Kerr and Dixon, 1974).

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Summary

1. The genital primordium gonads were examined at several stages during their development in the juvenile sea urchin.

2. At metamorphosis the primordial germ cells are a distinct group of cells in the dorsal mesentery, which also supports the stone canal and axial organs. They spread circumferentially along the aboral body wall to form a ring, the genital rachis, completely encircling the anal region. The gonads form as swellings in each interradius of this ring.

3. While the wall of the genitial primordium prior to rachis formation consists of a single layer of epithelial cells, that of the young gonad has two such layers separated by a fluid-filled space.

4. The primordial germs cells of each stage examined are similar in nuclear morphology, cytoplasmic inclusions, and cell shape and size. All stages have germ-cell-specific fibrillo-granular structures, which we name goniosomes.

5. A second cell type, the forerunner of the accessory cell of the mature gonad, is present in the genital tissue of all stages studied. Cellular inclusions characteristic of the accessory cell, including glycogen granules, lipid-like vesicles, and lysozymal-like vesicles, begin to accumulate in these cells even before gonad formation.

LITERATURE CITED

Akesson, B., 1961. A rapid method for orienting small and brittle objects for sectioning in definite planes. *Ark. Zool.*, **13**: 479–482.

Anderson, T. F., 1951. Techniques for the preparation of three dimensional structure in preparing specimens for the electron microscope. *Trans. N. Y. Acad. Sci.*, **13**: 130–134.

Bal, A. K., F. Jubinville, and G. H. Cousineau, 1969. Nuclear activity during oogenesis in sea urchins. II. Fine structural changes and patterns of RNA synthesis during meiotic prophases of *Arbacia punctulata* oocytes. *Z. Zellforsch. Mikrosk. Anat.*, **100**: 180–188.

Bal, A. K., F. Jubinville, G. H. Cousineau and S. Inoue, 1968. Origin and fate of the annulate lamellae in *Arbacia punctulata* eggs. *J. Ultrastruct. Res.*, **25**: 15–28.

Blackler, A. W., 1958. Contribution to the study of germ-cells in *Anura*. *J. Exp. Morphol.*, **6**: 491–503.

Chatlynne, L., 1969. A histological study of oogenesis in the sea urchin, *Strongylocentrotus purpuratus*. *Biol. Bull.*, **136**: 167–184.

Chatlynne, L., 1972. An ultrastructural study of oogenesis in the sea urchin *Strongylocentrotus purpuratus*. Ph.D. Dissertation, Oregon State University. 141 pp.

Cuenot, L., 1891. Etudes morphologiques sur les Echinodermes. *Arch. Biol.*, **11**: 313–380.

Davis, H. S., 1971. The gonad wall of Echinodermata: a comparative study based on electron microscopy. Masters Thesis, University of California, San Diego. 99 pp.

Delavault, R., 1966. Determinism of sex. Pp. 615–638 in R. A. Boolootian, Ed., *Physiology of Echinodermata*. Interscience Publishers, New York.

Delage, Y., and Hérouard, E., 1903, *Traite de Zoologie concrète les Echinoderms*. Schleicher Freres, Paris, 495 pp.

Dietrich, H. F., and A. R. Fontaine, 1975. A decalcification method for ultrastructure of echinoderm tissue. *Stain Technol.*, 50: 351–354.

Eddy, E. M., 1975. Germ plasm and the differentiation of the germ cell line. Pp. 229–280 in G. H. Bourne and J. F. Danielli, Eds., *International Review of Cytology*. Vol. 43. Academic Press, New York.

Gurr, E., 1965. P. 211 in *Rational Uses of Dyes in Biology*. Williams and Wilkins Co., Baltimore.

Hamann, O., 1887. Beitrage zure Histologie das Echinodermin. *Jena Z. Naturwiss.*, **21**: 87–262.

Hinegardner, R. T., 1969. Growth and development of the laboratory cultured sea urchin. *Biol. Bull.*, **137**: 465–475.

Holland, N. D., and A. C. Giese, 1965. An autoradiographic investigation of the gonads of the purple sea urchin (*Strongylocentrotus purpuratus*). *Biol. Bull.*, **128**: 241–258.

Kalt, M. R., 1973. Ultrastructural observations on the germ line of *Xenopus laevis*. *Z. Zellforsch. Mikrosk. Anat.*, **138**: 41–62.

Kawaguti, S., 1965. Electron microscopy on the ovarian wall of the echinoid with special reference to its muscles and nerve plexis. *Biol. J. Okayama Univ.*, **11**: 66–74.

Kerr, J. B., and K. E. Dixon, 1974. An ultrastructural study of germ plasm in spermatogenesis of *Xenopus laevis*. *J. Embryol. Exp. Morphol.*, **32**: 573–592.

Longo, F. L., and E. Anderson, 1969. Sperm differentiation in the sea urchins *Arbacia punctulata* and *Strongylocentrotus purpuratus*. *J. Ultrastruc. Res.*, **27**: 486–509.

MacBride, E. W., 1903. The development of *Echinus esculentus*, together with some points in the development of *E. miliaris* and *E. acutus*. *Philos. Trans. R. Soc. Lond. B. Biol.*, **195**: 285–327.

Mahowald, A. P., 1971. Polar granules of *Drosophila*. II. Ultrastructural changes during early embryogenesis. *J. Exp. Zool.*, **167**: 237–262.

Masuda, R., and J. C. Dan, 1977. Studies on the annual reproductive cycle of the sea urchin and the acid phosphatase activity of relict ova. *Biol. Bull.*, **153**: 577–590.

Miller, R. A., and H. B. Smith, 1931. Observations on the formation of the egg of *Echinodermata lucunter*. *Carnegie Inst. Wash. Publ.* No. 413: 47–52.

Millonig, G., M. Bosco, and L. Giambertone, 1968. Fine structure analysis of oogenesis in sea urchins. *J. Exp. Zool.*, **169**: 293–314.

Pearse, J. S., 1969a. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. I. The echinoids *Prionocidaris baculosa* (Lamarck) and *Lovenia elongata* (Gray). *Bull. Mar. Sci.*, **19**: 323–350.

Pearse, J. S., 1969b. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. II. The echinoid *Echinometra mathaei* (de Blainville). *Bull. Mar. Sci.*, **19**: 580–613.

Prouho, H., 1887. Recherches sur le *Dorocidaris papillata* et quelques autre echinoides de la Mediterranee. *Arch. Zool. Exp. Gen.*, **15**: 213–380.

Reynolds, E. S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.*, **17**: 208–213.

Russo, A., 1894. Sul sistema genitale e madreporico degli Echinidi regolari. *Boll. Soc. Nat. Napoli Ser. 1*, **8**: 90–107.

Spurr, A. R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, **26**: 31–43.

Verhey, C. A., and F. H. Moyer, 1967. Fine structural changes during sea urchin oogenesis. *J. Exp. Zool.*, **164**: 195–207.